Dynamic imaging of T cell surveillance in live tumor fragments using camelid nanobodies

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Introduction: Immunotherapies (ITx) have revolutionized the oncology landscape. However, predicting patient responses to ITx is difficult based solely on static correlates such as TIL localization and molecular signatures. Anti-tumor immune response depends on motile surveillance by tumor infiltrating lymphocytes (TIL) which recognize antigenic determinants and engage target cells in serial stop-and-go interactions that result in cell killing. However, the hostile tumor microenvironment (TME) can cause TIL dysfunction and lack of cytotoxicity which is manifested as either suppressed or aimless TIL motility. To determine which ITx works best for a given patient, we are developing a diagnostic platform using live tumor fragments (LTF) that preserves the TME and its immune cells. Antibody-based labeling of live tissues is hampered by slow diffusion and function-altering cross-linking. To overcome these limitations, we have used small camelid-derived monovalent antibodies(nanobodies) to monitor TIL motility in LTFs.

Methods: Human tumor excisions were cut into 300 x 300 x 200 µm LTFs, sorted into glass bottom multi-well plates and cultured. CD8+ cells in LTFs were labeled using an anti-hCD8a camelid VHH nanobody covalently labeled with AF594. The same reagent or a mouse anti-hCD8a whole IgG antibody was used to stain human peripheral blood mononuclear cells, and staining patterns were compared by flow cytometry. Immune responsiveness of LTFs to ITx was ascertained by flow cytometry and secreted protein assays (data shown in a companion abstract).

Results: Multiphoton microscopy revealed LTF collagen fibrils and cellular autofluorescence. A fluorescent anti-CD8a nanobody, but not a similarly labeled whole IgG, yielded good contrast and fast staining of two cell subsets. The smaller cells were 12 µm in diameter, cell surface-stained, and lacking autofluorescence, consistent with T cells. The larger cells were elongated, ramified, intracellularly stained, and distinctly auto fluorescent, consistent with macrophages. 3D motility tracking revealed characteristic translational motility of the smaller cells at ~10 µm/min along collagenous structures. In contrast, larger cells exhibited only slow motility.

Conclusion: Our results show that the motility of human CD8+ T lymphocytes can be revealed in LTF culture using a fluorescent CD8-binding camelid nanobody, likely due to its small size and monovalent binding. Further examination is needed to understand if T cell function is altered. The autofluorescence of larger, immotile cells was consistent with tumor-associated macrophages. Based on this distinction, T cells could be distinguished from the macrophages clearly. Our results support the use of camelid-derived VHH and other small monovalent reagents for live tissue lymphocyte tracking, possibly to evaluate TIL response to ITx in an LTF assay.

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